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(54) Method and formulation for orally administering bioactive agents to and through the Peyer's patch.

(57) A method and formulation for orally administering a bioactive agent which comprises encapsulating the agent in one or more biodegradable and biocompatible polymer or copolymer excipients to form a microcapsule which is capable of passing through the gastrointestinal tract unaffected and being taken up by the Peyer's patch.

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BACKGROUND OF THE INVENTION

This invention relates to a method and a formulation for orally administering a bioactive agent encapsulated in one or more biodegradable and biocompatible polymer or copolymer excipients which results in the agent reaching and being taken up by the folliculi lymphatic aggregati, otherwise known as the "Peyer's patch", of the animal without loss of effectiveness due to the agent having passed through the gastrointestinal tract.

The use of microencapsulation to protect sensitive bioactive agents from degradation has become well-known. Typically, a bioactive agent is encapsulated within a protective wall material, usually polymeric in nature. The agent to be encapsulated can be coated with a single wall of polymeric material, or can be homogeneously dispersed within a polymeric matrix. The amount of agent inside the microcapsule can be varied as desired, ranging from either a small amount to as high as 95% of the microcapsule composition. The diameter of the microcapsule can also be varied as desired, ranging from less than one micrometer to as large as three millimeters or more.

Peyer's patches are conglomerations of lymphoid nodules located in the ileum or lower part of the intestine, and are an important part of the body's defense against bacterial infection. Antigens are substances that promote antibody formation, and include such things as foreign protein or tissue. All antibodies belong to a class of proteins called immunoglobulins (Ig). When an antibody and antigen combine, they form an inactive complex, thus neutralizing the antigen.

Peyer's patches possess IgA precursor B cells which can populate the lamina propria regions of the gastrointestinal and upper respiratory tracts and differentiate into mature IgA synthesizing plasma cells. It is these plasma cells which actually secrete the antibody molecules. Studies by Heremans and Bazin measuring the development of IgA responses in mice orally immunized with antigen showed that a sequential apperance of antigen-specific IgA plasma cells occurred, first in mesenteric lumph nodes, later in the spleen, and finally in the lamina propria of the gastrointestinal tract (Brazin, H., Levi, G., and Doria, G. Predominant contribution of IgA antibody-forming cells to an immune response detected in extraintestinal lymphoid tissues of germfree mice exposed to antigen via the oral route. J. Immunol. 105: 1049; 1970 and Crabbe, P.A., Nash, D.R., Bazin. H. Eyssen, H., and Heremans, J.F. Antibodies of the IgA type in intestinal plasma cells of germfree mice after oral or parenteral immunization with ferritin. J. Exp. Med. 130 : 723; 1969). It is apparent, therefore, that Peyer's patches are enriched sources of precursor IgA cells, which, subsequent to antigen sensitization, follow a circular migrational pathway and account for the expression of IgA at distant mucosal surfaces. This circular pattern provides a common mucosal immune system by continually transporting sensitized B cells to mucosal sites for responses to gut-encountered environmental antigens and potential pathogens.

Of particular importance to the present invention is the ability of oral immunization to induce protective antibodies. It is known that the ingestion of antigens by animals results in the appearance of antigen-specific sIgA anti bodies in bronchial or nasal washings. For example, studies with human volunteers show that oral administration of influenza vaccine is effective at inducing secretory anti-influenza antibodies in nasal secretions.

It is apparent that any method or formulation involving oral administration of an ingredient be of such design that will protect the agent from degradation during its passage through the gastrointestinal tract. If not, the ingredient will reach the Peyer's patch, if at all, in inadequate quantity or ineffective condition. In unprotected form large quantities of the bioactive agent must be ingested for an effective amount of the agent to reach the Peyer's patch. The result is that a large percentage of the administered agent is unused. Also, frequent oral administrations are necessary to achieve a prolonged delivery of agent to the Peyer's patch. Such frequent administration of high doses of agent is both wasteful and inconvenient.

Therefore, there exists a need for a method of oral immunization which will effectively stimulate the mucosal immune system and overcome the problem of degradation of the bioactive ingredient during its passage through the gastrointestinal tract to the Peyer's patch. There exists a more particular need for a method of delivering an antigen to the Peyer's patch which does not result in degradation.

SUMMARY OF THE INVENTION

This invention relates to a method and formulation for delivering a bioactive agent to the Peyer's patch of an animal by oral administration. The agent is microencapsulated in a biodegradable and biocompatible polymer or copolymer which is capable of passing through the gastrointestinal tract without degradation or minimal degradation so that the agent reaches the Peyer's patch unaltered and in effective amounts. The term biocompatible is defined as a polymeric material which is not toxic to the body, is not carcinogenic, and should not induce inflammation in body tissues. The material should be biodegradable in the sense that it should degrade by bodily processes to products readily disposable by the body and should not accumulate in the body. The microcapsule is also of a size capable of being selectively taken up by the selective Peyer's patch. Therefore, the problems of the agent reaching the Peyer's patch and being taken up are solved.

It is an objective of this invention to provide a method of orally administering a bioactive ingredient to an animal which results in the ingredient reaching and being taken up by the Peyer's patch, and thereby stimulating the mucosal immune system, without losing its effectiveness as a result of passing through the animal's gastrointestinal tract.

It is a still further objective of this invention to provide a formulation consisting of a core bioactive ingredient and an encapsulating polymer or copolymer excipient which is biodegradable and biocompatible and which can be utilized in the oral administration methods described above.

DETAILED DESCRIPTION OF THE INVENTION

An illustration of the method of performing one embodiment of the invention, that is, the prolonged delivery of the antigen trinitrophenyl keyhole limpet hemocyanin encapsulated in 50:50 poly(DL-lactide-co-glycolide) to mice follows.

It should be noted, however, that other polymers besides poly(DL-lactide-co-glycolide) may be used. Examples of such polymers include, but are not limited to, poly(glycolic acid), copolymers of mixed DL-lactide and glycolide, copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(β-hydroxybutyric acid).

Also, other bioactive ingredients may be used. Examples of such, but not limited to include antigens to vaccinate against viral, bacterial, protozoan, fungal diseases such as influenza, respiratory syncytial, parainfluenza viruses, Hemophilus influenzae, Bordetella pertussis, Neisseria gonorrhea, Streptococcus pneumoniae andPlasmodium falciparum or other diseases caused by pathogenic microorganizms or antigens to vaccinate against dieseases caused by macroorganisms such as helminthic pathogens.

I. MICROENCAPSULATIONS

A. Preparation of Dye-Loaded Microcapsules for Peyer's-Patches Penetration Studies

Coumarin, a water-insoluble dye was microencapsulated with polystyrene, a nonbiodegradable polymer, to afford colorful microcapsules that could be used to follow the penetration of microcapsules into the Peyer's patches. The procedure used to prepare these microcapsules follows: First, a polymer solution is prepared by dissolving 4.95 g of polystyrene (Type 685D, Dow Chemical Company, Midland, MI) in 29.5 g of methylene chloride (Reagent Grade, Eastman Kodak, Rochester, NY). Next, about 0.05 g of coumarin (Polysciences, Inc., Warrington, PA) is added to the polymer solution and allowed to dissolve by stirring the mixture with a magnetic stir bar.

In a separate container, 10 wt % aqueous poly(vinyl alcohol) (PVA) solution, the processing medium, is prepared by dissolving 40 g of PVA (Vinol 205C, Air Products and Chemicals, Allentown, PA) in 360 g of deionized water. After preparing the PVA solution, the solution is saturated with methylene chloride by adding 6 g of methylene chloride. Next, the PVA solution is added to a 1-L resin kettle (Ace Glass, Inc., Vineland, NJ) fitted with a truebore stir shaft and a 2.5-in. teflon turbine impeller and stirred at about 380 rpm by a Fisher stedi speed motor.

The polystyrene/coumarin mixture is then added to the resin kettle containing the PVA processing media. This is accomplished by pouring the polystyrene/coumarin mixture through a long-stem 7-mm bore funnel which directs the mixture into the resin kettle. A stable oil-in-water emulsion results and is subsequently stirred for about 30 min at ambient pressure to afford oil microdroplets of the appropriate size.

Then the resin kettle is closed, and the pressure in the resin kettle is gradually reduced to 520 mm Hg by means of a water aspirator connected to a manometer and a bleed valve. The resin kettle contents are stirred at reduced pressure for about 24 h to allow all of the methylene chloride to evaporate. After all of the methylene chloride has evaporated, the hardened microcapsules are collected by centrifugation and dried for 72 h in a vacuum chamber maintained at room temperature.

B. Preparation of Antigen-Loaded Microcapsules

TNP-KLH, a water-soluble antigen, was encapsulated in poly(DL-lactide-co-glycolide), a biocompatible, biodegradable polyester. The procedure used to prepare the microcapsules follows:

First, a polymer solution was prepared by dissolving 0.5 g of 50:50 poly(DL-lactide-co-glycolide) in 4.0 g of methylene chloride. Next, 300 microliter of an aqueous solution of TNP-KLH (46 mg TNP-KLH/ml; after dialysis) was added to an homogeneously dispersed in the poly(DL-lactide-co-glycolide) solution by vortexing the mixture with a Vortex-Genie 2 (Scientific Industries, Inc., Bohemia, NY).

In a separate container, a 8 wt % aqueous PVA solution was prepared by dissolving 4.8 g of PVA in 55.2 g of deionized water. After dissolution of the PVA, the PVA solution was added to a 100-mL resin kettle (Kontes Glass, Inc., Vineland, NJ) fitted with a truebore stirrer and a 1.5-in. teflon turbine impeller. The polymer solution was then added to the PVA processing medium by pouring through a long-stêm 7-mm bore funnel. During this addition, the PVA solution was being stirred at about 650 rpm. After the resulting oil-in-water emulsion was stirred in the resin kettle for about 10 min, the contents of the resin kettle contents were transferred to 3.5 L of deionized water contained in a 4-L beaker and being stirred at about 800 rpm with a 2-in. stainless steel impeller. The resultant microcapsules were stirred in the deionized water for about 30 min, collected by centrifugation, washed twice with deionized water to removed any residual PVA, and were then collected by freeze drying. The microcapsule products consisted of spherical particles about 1 to 10 micrometer in diameter.

The TNP-KLH content of the antigen-loaded microcapsules, that is, the core loading of the microcapsules, was determined by weighing out 10 mg of antigen-loaded microcapsules in a 12-mL centrifuge tube. Add 3.0 mL of methylene chloride to the tube and vortex to dissolve the poly(DL-lactide-co-glycolide). Next, add 3.0 mL of deionized water to the tube and vortex vigorously for 1 min. Centrifuge the contents of the centrifuge tube to separate the organic and aqueous layers. Transfer the aqueous layer to a 10-mL volumetric flask. Repeat the extraction combining the aqueous layers in the volumetric flask. Fill the flask to the mark with deionized water. The amount of TNP-KLH in the flask and subsequently, the amount of TNP-KLH in the microcapsules is then quantified using a protein assay. The microcapsules contained 0.2 % TNP-KLH by weight.

II. BIOLOGICAL STUDIES

A. Mice

PALB/c mice, 8 to 12 weeks of age, were used in these studies.

B. Trinitrophenyl - Keyhole Limpet Hemocyanin

Hemocyanin from the keyhole limpet (KLH) Megathure crenulata was purchased from Calbiochem (San Diego, CA). It was conjugated with the trinitrophenyl hapten (TNP-KLH) using 2,4,6-trinitrobenzene sulfonic acid according to the procedure of Rittenburg and Amkraut (Rittenburg, M.B. and Amkraut, A.A. Immunogenicity of trinitrophenyl-hemocyanin: Production of primary and secondary anti-hapten precipitins. J. Immunol. 97: 421; 1966). The substitution ratio was spectrophotometrically determined to be TNP_{861}-KLH using a molar extinction coefficient of 15,400 at a wavelength of 350 nm and applying a 30% correction for the contribution of KLH at this wavelength (Rittenburg, M.B. and Amkraut, A.A. Immunogenicity of trinitrophenyl-hemocyanin: Production of primary and secondary anti-hapten precipitins. J. Immunol. 97: 421; 1966).

C. Immunization

Microencapsulated and nonencapsulated TNP-KLH was suspended at an antigen concentration of 10 µg/mL in a solution of 8 parts filter sterilized tap water and 2 parts sodium bicarbonate (7.5% solution). The recipient mice were fasted overnight prior to the administration of 0.5 mL of suspension via gastric intubation carried out with an intubation needle (Babb, J.L., Kiyono, H., Michalek, S.M. and McGhee, J.R. LPS regulation of the immune response: Suppression of immune responses to orally-administered T-dependent antigen. J. Immunol. 127: 1052; 1981).

D. Collection of biological fluids

1. Serum

Blood was collected in calibrated capillary pipettes following puncture of the retro-orbital plexus. Following clot formation, the serum was collected, centrifuged to remove red cells and plateletes, heat-inactivated, and stored at -70 °C until assayed.

2. Intestinal secretions

Mice were administered four doses (0.5 mL) of lavage solution (25 mM NaCl, 40 mM Na_2SO_4, 10 mM KCl, 20 mM $NaHCO_3$, and 48.5 mM polyethylene glycol, osmolarity of 530 mosM) at 15-min intervals (Elson, C.O., Ealding, W. and Lefkowitz, J. A lavage technique allowing repeated measurement of IgA antibody in mouse intestinal secretions. J. Immunol. Meth. 67: 101; 1984). Fifteen minutes after the last dose of lavage solution, the mice were anesthetized and after an additional 15 min they were administered 0.1 mg pilocarpine by ip injection. Over the next 10 to 20 min a discharge of intestinal contents was stimulated. This was collected into a petri dish containing 3 mL of a solution of 0.1 mg/mL soybean trypsin inhibitor (Sigma, St. Louis, MO) in 50 mM EDTA, vortexed vigorously and centrifuged to remove suspended matter. The supernatant was transferred to a round-bottom, polycarbonate centrifuge tube and 30 µL of phenylmethylsulfonyl fluoride (PMSF, Sigma) was added prior to clarification by high-speed centrifugation (27,000 x g, 20 min, 4 °C). After clarification, 20 µL each of PMSF and 1% sodium azide were added and the solution made 10% in FCS to provide an alternate substrate for any remaining proteases.

3. Saliva

Concurrent with the intestinal discharge, a large volume of saliva is secreted and 0.25 mL was collected into a pasteur pipette by capillary action. Twenty microliters each of soybean trypsin inhibitor, PMSF, sodium azide and FCS was added prior to clarification.

E. Immunochemical reagents

Solid-phase absorbed and affinity-purified polyclonal goat IgG antibodies specific for murine IgM, IgG and IgA were obtained commercially (Southern Biotechnology Associates, Birmingham, AL). Their specificity in radioimmunoassays were tested through their ability to bind appropriate monoclonal antibodies and myeloma proteins.

F. Solid-phase radioimmunoassays

Purified antibodies were labeled with carrier-free $Na^{125}I$ (Amersham) using the chloramine T method (Hunter, W.M. Radioimmunoassay. In: Handbook of Experimental Immunology, M. Weir (editor). Blackwell Scientific Publishing, Oxford. p. 14.1; 1978). Immulon Removawell assay strips (Dynatech) were coated with TNP conjugated bovine serum albumin (BSA) at 1 µg.mL in BBS overnight at 4 °C. Control strips were left uncoated but all strips were blocked for 2 h at room temperature with 1% BSA in BBS, which was used as the diluent for all samples and ^{125}I-labeled reagents. Samples of biologic fluids were diluted to contain 1 to

1,000 ng/mL of antigen-specific antibody of the isotype under study, added to washed triplicate replicate wells, and incubated 6 h at room temperature. After washing, 100,000 cpm of ^{125}I-labelled isotype-specific anti-immunoglobulin was added to each well and incubated overnight at 4 degree Centigrade. Following the removal of unbound ^{125}I-antibodies by washing, the wells were counted in a Gamma 5500 spectrometer (Beckman Instruments, Inc., San Ramon, CA). Calibrations were made using serial twofold dilutions of a standard serum (Miles Scientific, Naperville, IL) containing known amounts of immunoglobulins, on wells coated with 1 µg/well isotype-specific antibodies. Calibration curves and interpolation of unknowns was obtained by computer, using "Logit-log" or "Four Parameter Logistic" BASIC programs (RIA 001 and RIA 004) available from the Biomedical Computing Technology Center (Vanderbilt Medical Center, Nashville, TN).

G. RESULTS

1. Penetration of dye-loaded microcapsules into the Peyer's patches.

The uptake of microcapsules into the gut-associated lymphoreticular tissues and the size restriction of this penetration was investigated by orally administering to mice polystyrene microcapsules, loaded with the fluorescent dye coumarin. Unanesthetized, fasted BALB/c mice were administered 0.5 mL of a 100 µg/mL suspension of various sized fluorescent microcapsules (less than 5 µm or 8 to 50 µm in diameter) in tap water into the stomach using a feeding needle. At various times after administration (0.5, 1 and 2 h), the mice were sacrificed and the small intestine excised. One-cm sections of gut containing a descrete Peyer's patch were isolated, flushed of lumenal contents, everted, and snap frozen.

Frozen sections were prepared and examined under a fluorescence microscope to observe the number, location and size of the microcapsules which were taken up into the Peyer's patch from the gut lumen.

Although some trapping of the microcapsules between the villi had prevented their removal during flushing, no penetration into the tissues was observed at any point except the Peyer's patch. At 0.5 h after oral administration, microcapsules were observed in the Peyer's patch of the proximal, but not the distal, portion of the small intestine. With increasing time the microcapsules were transported by peristaltic movement such that by 2 h they were throughout the gastrointestinal tract and could be found in the Peyer's patch of the ileum. The endocytosed microcapsules were predominantly located peripherally, away from the apex of the Peyer's patch dome, giving the impression that physical trapping between the dome and adjacent villi during peristalsis had aided in their uptake. Although some particles up to 10 µm in diameter were observed within the Peyer's patch, microcapsules of less than 5 µm in diameter were taken up in greater numbers and were observed to progress deeper into the Peyer's patch over the time period examined. These results demonstrate that microcapsules of 1 to 5 µm in diameter are rapidly and selectively taken up from the gut lumen into the Peyer's patch. This suggested that microcapsules composed of biodegradable wall materials would serve as an effective means for the targeted delivery of antigens to the gut-associated lymphoreticular tissues for the induction of immunity at mucosal surfaces.

2. Oral immunization with antigen-loaded biodegradable microcapsules

Microcapsules containing the haptenated protein antigen trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) were prepared using poly(DL-lactide-co-glycolide) copolymers as wall materials. These microcapsules were separated according to size and those in the range of 1 to 5 µm in diameter were selected for evaluation. This microcapsules contained 0.2% antigen by weight. Their ability to serve as an effective antigen delivery system when ingested was tested by administering 0.5 mL of a 10 mg/mL suspension (10 µg antigen) in bicarbonate-buffered strerile tap water via gastric incubation on 4 consecutive days. For comparative purposes an additional group of mice was orally immunized in parallel with 0.5 mL of a 20-µg/mL solution of unencapsulated TNP-KLH. Control mice were orally administered diluent only.

On days 14 and 28 following the final immunization, serum, saliva and gut secretions were obtained from 5 fasted mice in each group. These samples were tested in isotype-specific radioimmunoassays to determine the levels of TNP-specific and total antibodies of the IgM, IgG and IgA isotypes (Table 1). The samples of saliva and gut secretions contained antibodies which were almost exclusively of the IgA class. These results are consistent with previous studies and provide evidence that the procedures employed to collect these secretions do not result in contamination with serum. None of the immunization protocols resulted in significant changes in the total levels of immunoglobulins present in any of the fluids tested. Low

but detectable levels of naturally-occurring anti-TNP antibodies of the IgM and IgG isotypes were detected in the serum and of the IgA isotype in the serum and gut secretions of sham innumized control mice. However, the administration of 30 µg of micro-encapsulated TNP-KLH in equal doses over 3 consecutive days resulted in the apperance of signficant antigen-specific IgA antibodies over controls in the secretions, and of all isotypes in the serum by Day 14 after immunization (see last column of Table 1). These antibody levels were increased further on Day 28. In contrast, the oral administration of the same amount of unencapsulated antigen was ineffective at inducing specific antibodies of any isotype in any of the fluids tested.

H. SIGNIFICANCE

These results are noteworthy in several respects. First, significant antigen-specific IgA antibodies are induced in the serum and mucosal secretions, a response which is poor or absent following the commonly used systemic immunization methods. Therefore, this immunization method would be expected to result in significantly enhanced immunity at the mucosa; the portal of entry or site of pathology for a number of bacterial and viral pathogens. Secondly, the microencapsulated antigen preparation was an effective immunogen when orally administered, while the same amount of unencapsulated antigen was not. Thus, the microencapsulation resulted in a dramatic increase in efficacy, presumable due to targeting of and increased uptake by the Peyer's patch. Thirdly, the inductive phase of the immune response appears to be of long duration. While systemic immunization with protein antigens in the absence of adjuvants is characterized by a peak in antibody levels in 7 to 14 days, the orally administered antigen-containing microcapsules induced responses which were higher at Day 28 than Day 14. This indicates that bioerosion of the wall materials and release of the antigen is taking place over an extended period of time, and thus inducing a response of greater duration.

TABLE 1. THE INDUCTION OF TNP-SPECIFIC ANTIBODIES IN THE SERUM AND MUCOSAL SECRETIONS OF BALB/C MICE BY ORAL IMMUNIZATION WITH MICROENCAPSULATED TNP-KLH

			ng Immunoglobulin/mL Sample					
			IgM		IgG		IgA	
Immunogen	Time After Immunization	Biologic Sample	Total	Anti-TNP	Total	Anti-TNP	Total	Anti-TNP
Control	Day 14	Gut wash	<1	<1	62	<1	79,355	25
		Saliva	<40	<10	<40	<10	2,651	<10
		Serum	445,121	6	5,503,726	37	1,470,553	32
Unencapsulated TNP-KLH	Day 14	Gut wash	4	1	131	<1	64,985	17
		Saliva	<40	<10	<40	<10	1,354	<10
		Serum	298,733	11	6,000,203	29	1,321,986	21
TNP-KLH Microcapsules	Day 14	Gut wash	3	<1	130	<1	95,368	222
		Saliva	<40	<10	<40	<10	1,461	88
		Serum	360,987	1,461	5,312,896	572	1,411,312	1,077
Unencapsulated TNP-KLH	Day 28	Gut wash	<1	<1	94	<1	88,661	64
		Saliva	<40	<10	<40	<10	1,278	<10
		Serum	301,223	21	5,788,813	67	1,375,322	63
TNP-KLH Microcapsules	Day 28	Gut wash	4	<1	122	2	82,869	422
		Saliva	<40	<10	<40	<10	1,628	130
		Serum	320,192	1,904	5,951,503	2,219	1,277,505	1,198

Claims

1. A method of orally delivering bioactive agent to the Peyer's patch of an animal, comprising the steps of:

(a) encapsulating effective amounts of said agent in a biodegradable and biocompatible excipient to form microcapsules of a size capable of passing through the gastrointestinal tract of an animal without degradation or with minimal degradation and being taken up by the Peyer's patch; and

(b) orally administering an effective amount of said microcapsules to the animal.

2. A composition to be orally administered to animals and capable of delivering a bioactive agent to the Peyer's patch of said animal, comprising an effective amount of a bioactive ingredient encapsulated in a biodegradable and biocompatible excipient so as to form microcapsules of a size capable of being taken up by the Peyer's patch and capable of passing through the gastrointestinal tract without degradation.

3. The composition as claimed in Claim 2, wherein said first and second copolymer excipients have different copolymer ratios.

4. The composition as claimed in Claim 3, wherein said microcapsule is 10 µm or less in diameter.

5. The composition as claimed in Claim 4, wherein said microcapsule is 5 µm or less in diameter.

6. The composition as claimed in Claim 4, wherein said microcapsule is 3 µm or less in diameter.

7. The composition as claimed in Claim 6, wherein said excipient is a polymer.

8. The composition as claimed in Claim 7, wherein said excipient is a copolymer.

9. The composition as claimed in Claim 8, wherein said excipient is selected from the group consisting of poly(glycolic acid), copolymers of mixed DL-lactide and glycolide, copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(B-hydroxybutyric acid).

10. The composition as claimed in Claim 9, wherein said copolymer excipient is poly(DL-lactide-co-glycolide).

11. The composition as claimed in Claim 10, wherein said copolymer excipient has mole rations of lactide to glycolide of 50:50 to 90:10, respectively.

12. The composition as claimed in Claim 2, wherein at least one said bioactive agent is protozoal, viral, fungal or bacterial antigen.

13. The composition as claimed in Claim 12, wherein said antigen is trinitrophenyl keyhole limpet hemocyanin.

14. The composition as claimed in Claim 13, wherein said microcapsules are of diameter from between 1 to 5 µm, said agent is trinitrophenyl keyhole limpet hemocyanin, and said excipient is 50:50 poly(D1-lactide-co-glycolide).

15. The composition as claimed in Claim 2, wherein said bioactive agent is a toxoid.

16. The composition as claimed in Claim 15, wherein said toxoid is a toxoid of a staphylococcal enterotoxin.